

NHANES 2001-2002 Data Release
May 2004
Documentation for Laboratory Results

Laboratory 3 – HIV antibody test result

(1) Documentation File Date – August 18, 2003

(2) Documentation File Name - Laboratory 3 – HIV antibody test result

(3) Survey Years Included in this File Release- 2001-2002

(4) Component Description

The estimated prevalence of human immunodeficiency virus (HIV) infection in the United States population is an important measure of the extent of the medical and financial burden the nation faces due to this virus. The current NHANES and HIV antibody data from NHANES III (1988-94) serves as a baseline for monitoring the changes in the epidemic over time in the general population of the United States. In addition to HIV antibody testing in NHANES, whole blood samples were collected and stored for future CD4 testing once the HIV status of the sample is known. This will allow CDC to determine the distribution of CD4 cells in a random sample of HIV positive individuals. NHANES is now the only national survey collecting blood on a population-based sample; therefore it will be a key element in future estimates. If the participant refused phlebotomy but did not refuse the HIV test urine was tested for HIV antibody.

(5) Sample Description:

5.1 Eligible Sample

Participants aged 18-49 years were tested.

(6) Description of the Laboratory Methodology

6.1 Serum assay

All specimens were tested using the Synthetic Peptide Enzyme Immunoassay (EIA) (Genetic SystemsTM HIV-1/HIV-2 Peptide EIA) for the detection of antibody to Human Immunodeficiency virus types 1 and/or2 (HIV-1 and HIV-2). (Bio-Rad Laboratories, Redmond, WA). Any specimen that reacted in an initial test was retested in duplicate with the Genetic SystemsTM HIV-1/HIV-2 Peptide EIA. Initially reactive specimens that were reactive in either one or both duplicates from the repeat testing are referred to as repeatedly reactive. These repeatedly reactive

specimens were then tested with a more specific test, using the Cambridge Biotech HIV-1 Western Blot Kit (Calypse Biomedical Corporation, Rockville, MD).

The combination of electrophoretic separation of complex mixtures of antigens with the highly sensitive immunoblotting technique has been useful in characterizing the antigenic profile of HIV-1 and describing the immune response to this virus in exposed or infected persons.

The Cambridge Biotech HIV-1 Western Blot Kit, when used as directed, will detect antibodies to HIV-1 when present in human serum or plasma. The position of bands on the nitrocellulose strips allows this antibody reactivity to be associated with specific viral antigens.

The Cambridge Biotech HIV-1 Western Blot Kit is manufactured by Calypse Corporation from HIV-I propagated in an H9/HTLV-IIIb T- Lymphocyte cell line. The partially purified virus is inactivated by treatment with psoralen and ultraviolet light, and detergent disruption. Specific HIV-1 proteins are fractionated according to molecular weight by electrophoresis on a polyacrylamide slab gel in the presence of sodium dodecylsulfate (SDS).

The separated HIV-1 proteins are electrotransferred from gel to a nitrocellulose membrane, which is then washed, blocked (to minimize nonspecific immunoglobulin binding), and packaged. Individual nitrocellulose strips are incubated with serum or plasma specimens, or controls. During incubation, if HIV-1 antibodies are present in the specimen, they will bind to the viral antigens bound to the nitrocellulose strips. The strips are washed again to remove unbound material.

Visualization of the human immunoglobulins specifically bound to HIV-1 proteins is accomplished in situ using a series of reactions with goat anti-human IgG conjugated with biotin, avidin conjugated with horseradish peroxidase (HRP), and the HRP substrate 4-chloro-1-naphthol. If antibodies are any of the major HIV-1 antigens are present in the specimen in sufficient concentration, bands corresponding to the position of one or more of the following HIV-1 proteins (p) or glycoproteins (gp) will be seen on the nitrocellulose strip: p17, p24, p31, gp41, p51, p66, gp120, gp160 (number refers to apparent molecular weight in kilodaltons).

6.2 Urine assay

Eligible individuals who refused phlebotomy or who did not have a sufficient blood sample for the serum HIV assay but who did not refuse HIV testing, had their urine tested for HIV Type 1 antibody using the CalypseTM HIV-1 Urine EIA. As with serum, initially reactive samples were retested in duplicate and all repeatedly reactive samples were confirmed with a Western Blot. The Cambridge Biotech HIV-1 urine Western Blot Kit (Calypse biomedical , Rockville, MD) was used to confirm

positive tests. The specifics of the urine assay are similar to the blood western blot (see above).

(7) Laboratory Quality Control and Monitoring

The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.

(8) Data Processing and Editing

Blood and urine specimens were processed, stored and shipped to the Division of AIDS, STD, and TB, National Center for HIV, STD, and TB Prevention, National Centers for Disease Control and Prevention. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Description of the Laboratory Methodology section.

(9) Data Access:

All data are publicly available.

(10) Analytic Notes for Data Users:

The serum specimens were first tested by enzyme immunoassay (EIA) and confirmed by the western blot (WB). If the EIA was repeatedly negative, the HIV antibody result was coded as negative. If the EIA was positive and the WB was positive the result was coded as positive. If the EIA was positive or indeterminate but the WB was negative the result was coded as negative. If the EIA was positive or indeterminate but the WB was indeterminate the result was coded as indeterminate.